

A METHOD FOR PRODUCING ANTERIOR ISCHEMIC OPTIC
NEUROPATHY IN ANIMALS

FIELD OF THE INVENTION

The present invention relates to a method to
5 determine the specific genes involved in damage to the
cells making up the axon fibers of the optic nerve, and
more particularly, to determine the changes in retinal
ganglion cell expression that occur in glaucoma and
ischemic optic neuropathy.

10 BACKGROUND OF THE INVENTION

Glaucoma and ischemic optic neuropathy are
leading causes of blindness in the United States, yet
despite the known histology and mechanics of the disease
(ischemic damage to the optic nerve, which includes the
15 axons of the retinal ganglion cells that synaptically
connect the retina to the central nervous system), the
specific genes involved in optic nerve maintenance and
axonal transport of factors supporting optic nerve function
are largely unknown. Glaucoma and ischemic optic
20 neuropathy are painless diseases, which involve damage to
the optic nerve and a chronic optic neuropathy. Research
has shown that heat shock Protein 90 (HSP90), a chaperone
protein has been shown to be intimately involved in retinal
ganglion cell (RGC) function, is present in high levels in
25 the optic nerve; the key site of glaucomatous and optic
neuropathic damage. Expression of chaperone proteins have
been associated with enhanced protection of retinal

ganglion cells, that are subjected to stresses similar to those found in ischemic optic nerve disease.

The use of this invention is to enable the examination *in vivo* of the changes in retinal ganglion cell gene expression that occur in glaucoma and optic neuropathy, using a low cost (rodent) animal model. There has recently been developed a new model of optic neuropathy in rat, which utilizes photosensitizing agents and irradiation of the optic nerve.

10 The chronic optic neuropathies selectively affect the retinal ganglion cell and its axon in an intact animal. Intact, adult, normal retinal ganglion cell function is part of a complex system consisting of full length, normal, differentiated retinal ganglion cells with their cell
15 bodies in the retina, and their longest cellular extensions (axons) synapsing in the central nervous system. The blood supply of the individual components (RGC body, axon, CNS termination) are also separate. Intact adult RGC's cannot be isolated or maintained in culture. Thus, the intact
20 adult retinal ganglion cell function cannot be studied in cell culture since it is part of a complex system. The majority of glaucoma studies and neuropathy studies have been conducted in rhesus monkeys, requiring the expenditure of large amounts of money and the sacrifice of previous
25 primates, as well as raising ethical considerations of primate usage. A new model of optic neuropathy in a rat, utilizing retinal laser treatment and photosensitization

has been developed in the present invention. The new model produces a potentially reversible, ischemic optic neuropathy. This method differs from the previous methods in that it is potentially reversible, is not induced by manual (incisional) surgery, and is variable in the level of severity that can be produced. The new model utilizes rat, and thus partially obviates the need for higher species (i.e., primates) in studying optic neuropathy and some forms of glaucoma.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 depicts the area of the eye affected by the laser and the effect produced by laser irradiation.

DETAILED DESCRIPTION OF THE INVENTION

Referring specifically to the single Figure, optic nerve 2 conveys visual information from the retina 4 to the brain for interpretation. Numeral 6 designates the sclera.

Rat (*Rattus Norvegicus*) are suitably anesthetized (typically with ketamine 35 mg/kg and xylazine 5 mg/kg) and the pupils of the eyes are dilated with an appropriate agent. After anesthesia, an intravenous injection of photosensitizing agent (in this case, Rose bengal, a fluorinated derivative of fluorescein) is administered through the tail vein. The choice of photosensitizing agent is based on the ability to generate free radical oxygenions on illumination. Dosed administration of the agent is typically given based on mg/kg animal weight. The

basis of the effect is not heat generation at the laser site, but rather a function of the effect that oxygen radicals have on damaging blood vessel endothelium inducing thrombosis and closure of the small vessels (capillaries) supplying the optic nerve head; at the junction of the retina and optic nerve.

One minute after the injection, a retinal fundus (standard opthamalic connotation for the retinal surface) contact lens that I have developed, that stabilizes the eye and allows direct visualization and photic treatment of the rat retina is applied to one eye, and the optic nerve is treated using a low intensity laser light; either argon laser light at 510nm to 565nm laser light with frequencies between 510nm and 565nm, or frequency doubled ND:YAG laser light at 535nm, a spot size as determined by the specimen, but nominally 500 microns size (must cover the optic nerve); laser power output power less than 0.010mW). A variety of laser frequencies, depending on the absorptive and emission spectrum of the dye compound may be used to generate oxygen free radicals. The treatment is continued for a suitable length of time to produce the desired extent of vessel damage and ischemic typically in the range of 8-40 seconds. Longer treatment (>25 seconds) is associated with central retinal artery blockage (retinal stroke).

One day after treatment, optic nerve ischemic can be visually identified by swelling of the optic nerve head, blurring of the optic disk margin, and pallor of the

treated region of the optic nerve head. Using electrophysiological means (visual evoked potential), optic nerve functional loss can be measured by a loss of VEP amplitude from 20-100%. A measure of the selectivity of the coupled photosensitizing agent--laser treatment in producing optic neuropathy can be determined by laser illumination of the optic nerve in animals that are not injected with photosensitizing agent. Typically the control eyes show little or no change in gross features, histological features, and electrophysiological (VEP) measures.

Animal VEP's are performed using standard electrophysiological techniques. The head of the animal is shaved and electrode paste is applied to two sites on the
15 animals head. Electrical transmission along the optic nerve is measured and amplified. After eliciting the VEP, descriptive statistical analysis is used to compare the results of the VEP tests. Ocular tissues of the treated and control animals are also examined histologically.

20 BRIEF DESCRIPTION OF THE PRESENT INVENTION

In accordance with the present invention, recently there has been developed a new model of optic neuropathy in rat utilizing retinal laser treatment combined with photochemical-induced closure of selected
25 vessels (capillaries) of the optic nerve. This model is useful in studying *in vivo* optic neuropathy and the

physiological responses to neuropathy in the intact organism.

The treatment contemplated herein damages only small capillaries and the purpose of the treatment is described above. Specifically, the treatment is the exposure to laser light of the optic nerve of the specimen. The exposure as indicated above is to affect only the capillaries supplying the optic nerve. Ancillary damage must be minimized.

10 The result of the above is that there is now a model that grossly and histologically resembles anterior, ischemic optic neuropathy that looks like the human optic nerve disease encountered in anterior, ischemic optic neuropathy, glaucoma, and related eye disease. There is a
15 block of much of optic nerve transport one day after a single optic nerve vessel treatment. After awhile, about 40% of retinal transport cells die. Experiments can now be conducted on the rat to attempt to locate the specific genes involved so they can be treated to cure such
20 diseases, and in particular, anterior ischemic optic neuropathy and glaucoma. The experiments concern an attempt to prevent occurrence of severe damage to the optic nerve.

Once given the above disclosure, many other
25 features, modifications and improvements will become apparent to the skilled artisan. Such features, modifications and improvements are, therefore, considered

